Hedgehog and retinoic acid signaling cooperate to promote motoneurogenesis in zebrafish.

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Public Summary:
During early vertebrate development, the central nervous system (CNS) arises through the creation, organization, and coordination of many different cell types. One great challenge in biology is to identify the elementary steps of this process, and the molecular signals that control them. Successful neural stem cell-based regenerative therapies will directly require this knowledge, since neural stem cell activity recapitulates neurodevelopment in many aspects. Over the last 10-20 years, a number of the key signals in this process have been identified. One is the Hedgehog family of secreted proteins, which instructs undifferentiated neural progenitor cells to acquire cell fates of the ventral CNS (for example motoneurons which project from the spinal cord to skeletal muscle to control body movement). A second key signal in CNS development is the chemical retinoic acid (RA), which promotes some hindbrain and spinal cord CNS cell fates, particularly motoneurons. In order to interrogate how Hedgehog signaling and RA work together to control motoneuron differentiation in the nascent spinal cord, we sought to eliminate both Hedgehog signaling and RA during embryogenesis to assess the effects on motoneuron development. For these experiments we used zebrafish embryos, since their motoneurons and motoneuron activity (muscle twitching) are easily observed, and since it is possible to eliminate both Hedgehog signaling and RA in these embryos. We first generated MZsmo mutant embryos, which lack a transmembrane protein required for all cellular responses to the Hedgehog signal. These embryos exhibit fewer motoneurons and weaker motoneuron activity but some motoneurons still form. Genetic ablation of two transcription factors within the Hedgehog pathway, Gli1 and Gli2b, caused further reductions in motoneuron numbers. These results demonstrate that even in the absence of Hedgehog signals, other Hedgehog pathway components are important for cell fate determination. Next, we assessed the role of RA in motoneuron formation using pharmacological tools. MZsmo embryos treated with a chemical inhibitor of RA synthesis lose their remnant motoneurons. These observations imply that RA cooperates with Hedgehog signaling to drive motoneuron fate determination in the living CNS. We concluded our studies by examining how RA and Hedgehog signaling work together to drive motoneurogenesis. Both pathways had similar effects on cell division and activation, suggesting common mechanisms of action. In addition, RA affected Hedgehog pathway activity and Gli2b levels within the developing spinal cord. Together these results suggest the motoneurogenic activity of RA is due at least in part to its ability to modulate Hedgehog pathway components. Our studies illustrate how two developmental signals can cooperatively control neural cell fate decisions such as motoneuron formation. A comprehensive understanding of spinal cord development will require an integrative view of multiple signaling pathways in the manner we have done so here, and which will be essential for harnessing the regenerative capabilities of neural stem cells for CNS injury therapy.

Scientific Abstract:
The precise requirements of Hedgehog (Hh) pathway activity in vertebrate central nervous system development remain unclear, particularly in organisms with both maternally and zygotically derived signaling. Here we describe the motoneural phenotype of zebrafish that lack maternal and zygotic contributions of the Hh signaling transducer Smoothened (MZsmo mutants) and therefore are completely devoid of ligand-dependent pathway activation. Some functional primary motoneurons (PMNs) persist in the absence of Hh signaling, and we find that their induction requires both basal Gli transcription factor activity and retinoic acid (RA) signaling. We also provide evidence that RA pathway activation can modulate Gli function in a Hh ligand-independent manner. These findings support a model in which Hh and RA signaling cooperate to promote PMN cell fates in zebrafish.